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Natural and Environment-friendly New Bactericide for Leather Industry: Essential Oil of *Origanum minutiflorum*

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Abstract: In this study, essential oil of *Origanum minutiflorum* was purchased and used as a bactericide during soaking process to avoid bacterial growth. One gram per liter of oil was applied and also commercial bactericide which is commonly used in leather industry, 7-25% phenol, 4-chloro-3-methyl was used and compared. The trials showed that *Origanum minutiflorum* has much better antibacterial effect than commercial bactericide. From the study it was concluded that this essential oil can be used as bactericide in leather industry.

Key words: *Origanum minutiflorum*, pickle, wet blue, bactericide, essential oil

INTRODUCTION

Soaking is the first process which rehydrates and cleans hides or skins. The raw materials of the tanners are not sterile^[1]. When the soaking process starts the bacteria whose activities are restricted by good conservation start to grow. In fact, for a short soaking period bacteria don't cause important defects but when the skins or hides are too dry, they need long soaking periods. Longer soaking periods result in great microbial growth and sometimes this may also cause permanent damages. The researchers agreed that a wide variety of bacteria have been isolated from the soak water, including species of *Bacillus*, *Chromobacter*, *Pseudomonas*, *Clostridium*, *Lactobacillus* and *Serratia marcescens*^[2]. When the bacteria eating up the hides putrefactive odors and hair slip can be seen. If the bacterial damage was too much, tiny abrasions, known as 'pin prick' damaging the grain of leather may come out^[3]. Moreover damage on epidermis layer, matt and lusterless grain, loss of the skin substance, etc. can occur.

For longer soaking process it should be better to use bactericide to prevent these damages. But the chemicals which are used for this purpose are generally harmful to human health and nature. Furthermore, the use of these kinds of chemicals are either restricted or banned by some countries. Due to restrictions and use of lesser biocide than necessary, some microorganisms can develop resistance to microbicides^[4]. So, leather market needs new biocides which doesn't give harm to human health and natural life. In this study, a completely natural product was used as a bactericide and compared with commercial one.

The antimicrobial activity of plant essential oils and their derivatives have been recognized long time ago. İbn-I Sina was the first man who invented the essential oils by steam distillation techniques and used (980-1037)^[5]. Essential oils and their components are becoming increasingly popular since they are natural products and such antimicrobial agents have been used in many different sectors such as pharmacy, bakery etc. In this study, essential oil of *Origanum minutiflorum* which endemic in Türkiye was used. Essential oil from the oregano plant is produced by steam distillation of the leaves and flowering tops. Oregano oil contains four main groups of chemicals that contribute to its potent healing powers. Phenols, such as carvacrol and thymol, act as antiseptics and antioxidants, while terpenes, which include pinene and terpinene, exhibit antiseptic, antiviral, anti-inflammatory and anesthetic properties. Linalool and borneol are two long-chain alcohols found in oregano oil, which add to the antiviral and antiseptic agents^[6]. The objective of this study was to determine if the essential oil of *Origanum minutiflorum* can be used as an alternative to bactericides in the soaking process and to compare it with a widely used commercial bactericide.

MATERIALS AND METHODS

Materials

Rawskins: In this study, dry salted domestic sheep skins were used.

Essential oil: *Origanum minutiflorum* is endemic species in Türkiye. The essential oil was purchased from a commercial company.

Table 1: Chemical constituents of the essential oils of *Origanum minutiflorum* (TBAM 005/2001)

Constituents	<i>O. minutiflorum</i> essential oil (%)
Carvacrol	78.8
γ -terpinen	3.7
p-cimen	3.5
β -cariofillen	1.9
α -pinene	1.3
Myrsen/ α -phellandren	1.1
α -terpineol/borneol	1.1
Thymol	1.1

Table 2: Soaking processes

Experiments
Soaking with bactericide (24 h)
% 1000 water 28°C
%1 Bactericide (1 g L ⁻¹)
Soaking with <i>O. minutiflorum</i> (24 h)
% 1000 water 28°C
%1 <i>Origanum minutiflorum</i> essential oil (1 g L ⁻¹)
Control (24 h)
% 1000 water 28°C

Although there is a combined effect of the many compounds found in oregano oil, its effectiveness is largely attributed to its primary ingredient, a phenol named carvacrol. When Lab tested, carvacrol was found to be one of the most potent antibiotics known to science. In tests done at Georgetown University, it was found to be more effective than penicillin, streptomycin and even vancomycin (considered to be the strongest of all antibiotics)^[6].

The essential oil's chemical structure and the percentage of constituents are given Table 1. This percentage were given Atatürk University (TBAM) which report number was 005/2001.

Bactericide: The commercial bactericide, commonly used in leather industry, 7-25% phenol, 4-chloro-3-methyl was used.

Media: In microbiological studies, PCA (Plate Count Agar) (Oxoid) media was used to count the total aerobic mezophyl bacteria.

Methods

Soaking process: In experiments, 1 g L⁻¹ *Origanum minutiflorum* essential oil and 1 g L⁻¹ bactericide were used in recommended amount by the commercial company. Essential oils are not soluble in water. For this reason, it was diluted with ethanol 1/1 (v/v) then it was poured into the main soaking liquer. This diluted oil was used for a 24 h soaking period following the 2 h presoaking process. The objective of presoaking process was to remove materials like conservation salt, dust and dung on the skins. After 24 h, the samples were taken from the floats and microbiological study was carried out

in order to determine the amount of bacteria. The same microbiological tests without protective chemicals were also made for the samples which were taken from the soak water and the amount of bacteria were determined (Table 2).

Microbial counting method: The Total Aerobic Mezophyl Bacteria were counted in the media Plate count agar (PCA-Oxoid). The incubator temperature was regulated at 37°C. At the end of 48 h the amount of alive microorganisms in the soaking process was calculated from the colony number found in petri dishes and the results are reported as colony forming unit in millilitre (cfu mL⁻¹)^[3]. The experiments repeated 5 times and every experiment had 2 parallel.

RESULTS AND DISCUSSION

As seen from Table 3, it was found that after 24 h soaking period, *Origanum minutiflorum* has much better antibacterial effect than commercial bactericide. As a rule of thumb, one of the big company (Buckman) where produce biocide has always maintained that bacterial population of above 10⁵ cfu mL⁻¹ could be problematic. Clearly it would be ideal to have populations less than 10⁵ cfu mL⁻¹^[6]. This finding means that the use of this oil as a bactericide for soaking process in leather industry is possible. The total aerobic mezophyl bacterial count in the soaking process consisting of 1g L⁻¹, *Origanum minutiflorum* essential oils, 1g L⁻¹ bactericide and control are given in Fig. 1. There could be some bacteria more resistant to essential oil and still alive. In general, Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria^[7]. Some of the researchers also investigated the activity of the essential oil against *Aeromonas hydrophila*, *Bacillus amyloliquefaciens*, *B. brevis*, *B. cereus*, *B. subtilis*, *Corynebacterium xerosis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Staphylococcus aureus* and *Yersinia enterocolitica*, using a paper disc diffusion method and reported that *Origanum minutiflorum* essential oils inhibited all bacteria at concentrations of <1/100 (v/v)^[8]. Also the growth of *Salmonella enteritidis* was inhibited by the essential oil of oregano^[9].

As a result of this study, it was concluded that *Origanum minutiflorum* essential oil can be used as a new bactericide which doesn't give harm to nature and human health since it is completely natural. Moreover unlike pharmaceutical antibiotics, oregano oil does not create resistant strains of mutant bacteria^[6]. It is able to naturally

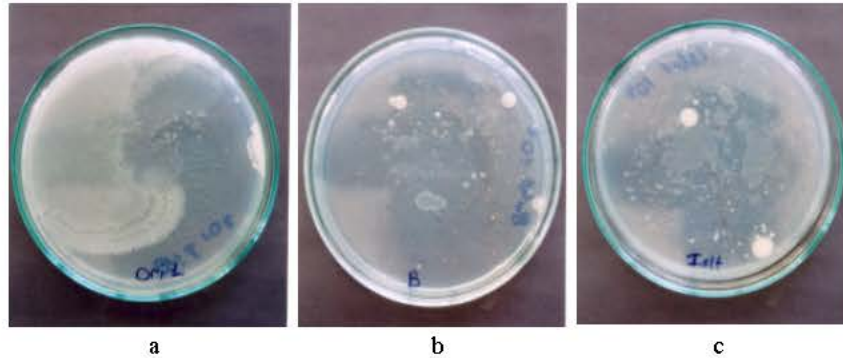


Fig. 1: The total aerobic mezophyl bacterial count in the soaking process a) 1 g L^{-1} *Origanum minutiflorum* essential oil, b) 1 g L^{-1} Bactericide and c) is control.

Table 3: The experiment results after 24 h soaking process period

Experiments (24 h)	Total aerobic mezophyll bacteria amount (cfu mL ⁻¹)	Temperature	
		pH	(°C)
Bactericide (1 g L^{-1})	1.83×10^6	8	22
<i>Origanum minutiflorum</i> essential oil (1 g L^{-1})	7.4×10^2	8	22
Control	3×10^6	8	22

and effectively, eliminate any pathogen that is unfortunate enough to cross its path.

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